

CLAIMS

What is claimed is:

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1. A method for generating an infectious clone based on the genome of a positive strand RNA virus, said method comprising producing a recombinant nucleic acid comprising at least one full-length DNA copy or in vitro-transcribed RNA copy or a derivative of either whereby the RNA virus has a genome of at least about 15 kb.
2. A method for generating an infectious clone based on the genome of an RNA virus, said method comprising producing a recombinant nucleic acid comprising at least one full-length DNA copy or in vitro-transcribed RNA copy or a derivative of either and further comprising selecting infectious clones by transfecting a host cell with said recombinant nucleic acid whereby said host cell is in essence not susceptible to infection with said virus.
3. A method according to claim 2 whereby said virus is a positive strand RNA virus with a genome of at least about kb.
4. A method according to claim 2 or 3 whereby said host cell is a BHK-21 cell
5. A recombinant nucleic acid comprising an infectious clone obtainable by a method according to any one of claims 1 to 4
6. A recombinant nucleic acid according to claim 5 comprising an infectious clone based on the genome of a virus selected from any of the Nidovirales.
7. A recombinant nucleic acid according to claim 6 comprising an infectious clone based on the genome of a virus selected from any of the Artiriviridae.
8. A recombinant nucleic acid according to claim 7 wherein said virus is PRRSV.

9. A recombinant nucleic acid molecule according to any of claims 5 to 8 wherein a nucleic acid sequence encoding a virulence marker and/or a serological marker has been modified.
10. A recombinant nucleic acid molecule according to claim 9 wherein the nucleic acid sequence encoding said marker is located within any of the open reading frames encoding structural viral proteins.
11. A recombinant nucleic acid molecule according to claim 10 wherein one open reading frame is ORF7 of any of the Arteriviridae.
12. A recombinant nucleic acid molecule according to any of claims 5 to 8 wherein one open reading frame is substituted by an ORF7 of the Arteriviridae.
13. A recombinant nucleic acid molecule according to any of claims 5 to 12 wherein at least one additional heterologous nucleic acid sequence is inserted.
14. A recombinant nucleic acid molecule according to claim 13 wherein said heterologous nucleic acid sequence encodes an antigen.
15. A recombinant nucleic acid molecule according to any of claims 5 to 14 wherein an open reading frame has been modified.
16. A modified RNA virus comprising a recombinant acid according to any of claims 5 to 15.

17. A vaccine comprising a modified RNA virus according to claim 16.
18. A cell infected with a modified RNA virus according to claim 16.
19. A protein and/or antigen obtained from a cell culture according to claim 18.
20. A diagnostic assay using a protein and/or antigen according to claim 19.
21. An isolated nucleic acid comprising a DNA sequence encoding an infectious RNA molecule encoding a North American PRRS virus, wherein said DNA sequence is SEQ ID NO:24 or a sequence that hybridizes to the complement of SEQ ID NO:24 under conditions comprising hybridization to filter-bound DNA in 0.5 M NaHPO₄, 7% SDS, 1 mM EDTA at 65 °C, and washing in 0.1X SSC/0.1% SDS at 68 °C.
22. A transfected host cell comprising a DNA sequence encoding an infectious RNA molecule encoding a North American PRRS virus, wherein said DNA sequence is SEQ ID NO:24 or a sequence that hybridizes to the complement of SEQ ID NO:24 under conditions comprising hybridization to filter-bound DNA in 0.5 M NaHPO₄, 7% SDS, 1 mM EDTA at 65 °C., and washing in 0.1X SSC/0.1% SDS at 68 °C, which transfected host cell is capable of expressing the encoded North American PRRS virus.
23. An isolated nucleic acid comprising a DNA sequence encoding an infectious RNA molecule encoding a North American PRRS virus, wherein said DNA sequence is SEQ ID NO: 24.
24. An isolated nucleic acid in the form of a plasmid, wherein said isolated nucleic acid comprises a DNA sequence encoding an infectious RNA molecule encoding a North American PRRS virus, wherein said DNA sequence is SEQ ID NO: 24.

25. An isolated infectious RNA molecule encoded by an isolated nucleic acid comprising SEQ ID NO: 24, which infectious RNA molecule encodes a North American PRRS virus.

26. A recombinant North American PRRS virus encoded by an isolated nucleic acid comprising a DNA sequence encoding an infectious RNA molecule encoding a North American PRRS virus, wherein said DNA sequence is SEQ ID NO: 24.